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Simultaneous determination of albendazole and its principal metabolites in plasma by normal phase high-performance liquid chromatography*

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Abstract: A sensitive, specific and reproducible high-performance liquid chromatography procedure using normal phase is described for the simultaneous determination of albendazole, albendazole sulphoxide and albendazole sulphone in sheep plasma, with mebendazole as an internal standard. Analysis of plasma requires only 100 μl of sample, which is extracted with ethylacetate and injected directly onto a 5-μm normal phase column, using hexane–ethanol (445:55, v/v) as eluent, with detection at 225 nm. The standard curves in plasma were linear for both albendazole and its metabolites at concentrations from 0.1 to 10 μg/ml. The method has been applied to the determination of plasma levels of albendazole and metabolites during preliminary pharmacokinetic studies in sheep.

Keywords: *High-performance liquid chromatography; albendazole; sulphoxide and sulphone metabolites; normal phase; pharmacokinetics; sheep.*

Introduction

Albendazole is reported to be an effective anthelmintic agent against liver flukes, tapeworms and lung and gastro-intestinal nematodes in farm animals [1]. More recently, its clinical use in man has been described for hydatide disease [2]. Albendazole is a potent member of the benzimidazole group of anthelmintics which act by inhibiting the uptake of glycogen in the gut of parasites or by inhibiting the enzyme fumarate reductase [1].

Metabolic studies of albendazole using radiolabelled compound in domestic animals [3] have described the transformation of the product, and showed the rapid appearance of two sulphoxide metabolites: albendazole sulphoxide and albendazole sulphone (Fig. 1). A previous study [4] indicated that other similar thio-compounds with anthelmintic properties such as bithionol, phenothiazine or fenbendazole were similarly oxidized to sulphoxides. Furthermore, it appears that the specific activity of albendazole is probably attributable to albendazole sulphoxide [2]. Accordingly, pharmacokinetic studies require

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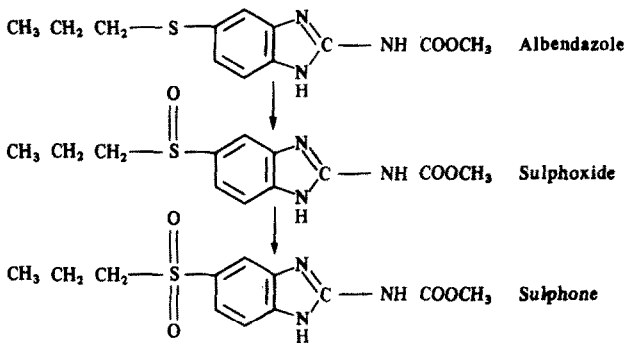

Figure 1
Chemical structure of albendazole and its principal metabolic products.

an analytical method which permits simultaneous monitoring of the parent compound and its two major metabolites, sulphoxide and sulphone.

An original technique for the determination of benzimidazole products such as albendazole in biological fluids, using reversed-phase high-performance liquid chromatography (HPLC) and a basic eluent, has been described [5]. However this technique does not report the levels of the individual metabolites. The same authors have described the disposition of albendazole in sheep, using a modification of their earlier technique requiring two successive reversed-phase chromatographic assays, to resolve the drug and its sulphoxide and sulphone metabolites [6]. These procedures are inconvenient in that they are time-consuming and require the use of two different columns. Furthermore, no internal standard was used in the published procedure.

The purpose of the present work is to describe a sensitive and specific method which permits the simultaneous determination of albendazole and its sulphoxide and sulphone metabolites. The combination of a simple extraction procedure with a highly efficient separation based on normal phase chromatography and an internal standard, offers a practical method for pharmacokinetic studies.

Materials and Methods

Chemicals and reagents

All reagents were of analytical grade purity. Ethanol was purchased from Merck (Darmstadt, FRG). Hexane and ethylacetate originated from Solvent Documentation Synthèse Co (Peypin, France). Water was deionized and distilled. Albendazole and its sulphoxide, sulphone and amino-sulphone were kindly provided by Dr M. Chaton-Schaffner (Smith-Kline Laboratoires, Paris, France). Mebendazole was purchased from Dr L. Ooms (Janssen Pharmaceuticals, Brussels, Belgium).

Chromatographic system

Separation was performed using a liquid chromatograph model 202 (Waters Associates, Milford, MA, USA) equipped with a Waters M600 A solvent delivery system and a Waters U6K universal injector. The column (250 × 4.6 mm i.d.) consisted of a normal phase Partisil 5-μm packing (Whatman SA, Paris, France). The eluate was monitored UV-spectrophotometrically at 225 nm using a model SH 770 detector (Schoeffel Instruments, West Wood, NJ, USA).

The mobile phase comprised hexane–ethanol (445:55, v/v). This solution was passed through a 0.45-μm filter (Whatman, Maidstone, UK) and degassed under vacuum and prepared fresh daily. The flow rate was maintained at 1.5 ml/min, giving a back-pressure of 1000 p.s.i. at room temperature.

Preparation of standard solutions

The solutions of albendazole, sulphoxide and sulphone used to construct the standard curve were prepared by serial dilution of a stock solution containing 1.0 mg/ml in methanol. A stock solution of the internal standard (mebendazole) was prepared in methanol (1 mg/ml). A working standard was prepared by diluting 1.0 ml of this solution to 50.0 ml with methanol to give a solution containing 20 μg/ml.

Extraction procedure

100 μl of plasma or whole blood, 10 μl of the working solution of internal standard (equivalent to a 0.2 μg mebendazole), and 1.0 ml of ethyl-acetate were added to a 1.5 ml polypropylene tube. The samples were shaken on a table-top shaker for 15 min and centrifuged for 2 min at 5000 *g*. The organic layer was aspirated, transferred to a 15-ml centrifuge tube and evaporated to dryness under a stream of nitrogen gas to prevent oxidation. The sample extract was reconstituted with 50 μl of eluent, mixed using a vortex mixer and the total then injected onto the column.

Calibration and reproducibility

Pooled blank sheep plasma samples were spiked with albendazole, albendazole sulphoxide or albendazole sulphone to give levels from 0.1 to 10 μg/ml. A constant amount (10 μl) of working solution of the internal standard was added to each sample. Pooled plasma samples were run through the procedure and calibration curves constructed by plotting the peak height ratio for each compound with respect to the internal standard, against the amount of compound added to each blank plasma sample. Peak heights were measured to the extrapolated trailing-edge baseline.

Least-squares regression analysis was used to determine the slope, intercept and correlation coefficient for each compound over two concentration ranges (Table 1). The

Table 1
Least-squares regression statistics for HPLC calibration data of albendazole and its sulphoxide and sulphone metabolites in spiked samples of sheep plasma, using mebendazole as internal standard

	Range 0.1–1 μg/ml (0.04 a.u.f.s.)			Range 0.1–10 μg/ml (0.4 a.u.f.s.)		
Component*	*m*†	*c*‡	*r*§	*m*	*c*	*r*
Albendazole	0.991	−0.0093	0.997	1.01	−0.0910	0.999
Albendazole sulphone	0.486	−0.0058	0.998	0.501	0.017	0.999
Albendazole sulphoxide	0.375	0.0082	0.998	0.382	0.022	0.999

* Plasma samples were spiked by adding the component dissolved in methanol.
† *m* Gradient.
‡ *c* Intercept.
§ *r* Correlation coefficient ($n = 6$).

response of the HPLC system was linear from 0.1–1 μg/ml and from 0.1–10 μg/ml for all three compounds.

Extraction-recovery

The extraction-recovery of the benzimidazoles was measured by comparing the chromatographic peak height for spiked plasma with that obtained by direct injection of standard in methanol. Extraction-recoveries of albendazole, sulphone and sulphoxide were 78.2, 81.2 and 84.2% respectively, with relative standard deviations of 7.5, 3.53 and 3.74% (n = 5), respectively, for an initial level of 1.0 μg/ml for each compound. These results are in agreement with findings of other workers [6].

Recovery and precision

The recovery and precision of the procedure was ascertained by adding 1.0 μg/ml each of albendazole, sulphoxide and sulphone to drug-free sheep plasma and analysing six samples by the proposed method. The results are summarized in Table 2.

Retention time and selectivity

Normal phase chromatography using silica gel with a nominal particle size of 5 μm showed high selectivity in the separation of the benzimidazoles within 15 min, as shown in Table 3.

Table 2
Recovery and precision of the HPLC method for the determination of albendazole and its metabolites in sheep plasma samples

Theoretical concentration (1.00 μg/ml)	Concentration found ± SEM*	Recovery (%) †	RSD (%) ‡
Albendazole	0.989 ± 0.041	98.9	4.15
Sulphone	1.046 ± 0.030	104.6	2.94
Sulphoxide	0.983 ± 0.024	98.3	2.44

* SEM, standard error of the mean (n = 6).
† Recovery expressed relative to theoretical spiked concentration.
‡ RSD, Relative standard deviation.

Table 3
Retention times and capacity factors of benzimidazoles studied

Compound	Capacity factor (k')	Retention time* (min)
Albendazole	1.2	4.4
Mebendazole	2.4	7.0
Albendazole sulphone	4.4	11.0
Albendazole sulphoxide	6.6	15.0

* Column hold-up time was 2.10 min.

Drug disposition study

To test the ability of this method to detect albendazole and its metabolites during pharmacokinetic studies, albendazole was administered intravenously to a sheep at a dose-rate of 1.9 mg/kg. Blood was withdrawn via the jugular vein at 15 and 30 min and at 1, 2, 4, 6, 8, 11 and 24 h after injection and transferred to tubes containing 10 U of heparin. The plasma was separated immediately and stored at −20°C until analysis.

Results and Discussion

Chromatography

A chromatogram of a mixture of 0.1 μg of albendazole, mebendazole, albendazole sulphone and albendazole sulphoxide is shown in Fig. 2a. This chromatogram illustrates the clear separation of the benzimidazoles using the method described. Figure 2b shows the chromatogram of a blank sheep plasma spiked with internal standard at 2.0 μg/ml. Figure 2c displays a chromatogram of plasma spiked with 1.0 μg each of albendazole, sulphone, sulphoxide and mebendazole.

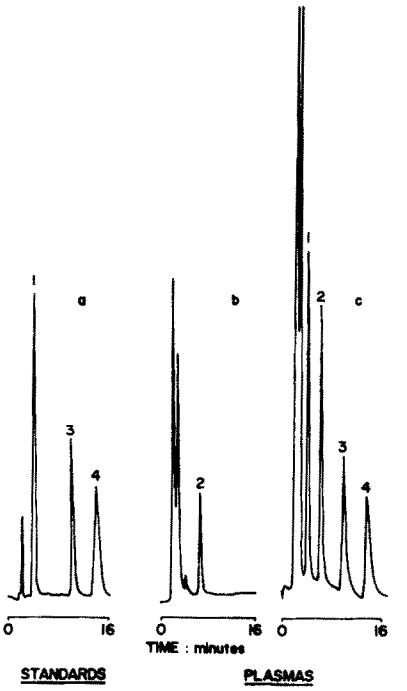

Figure 2
High-performance liquid chromatography of benzimidazoles.
(a) Separation of a standard benzimidazole mixture in methanol (100 ng of each): 1 = albendazole, 3 = albendazole sulphone, 4 = albendazole sulphoxide; detection sensitivity 0.04 a.u.f.s. at 225 nm.
(b) Chromatogram showing an extract of sheep plasma spiked with internal standard (2, mebendazole) at 2.0 μg/ml (0.1 a.u.f.s.).
(c) Chromatogram of sheep plasma extract spiked with 1 μg/ml each of: 1, albendazole; 2, mebendazole; 3, albendazole sulphone; 4, albendazole sulphoxide; detection sensitivity 0.04 a.u.f.s.

In order to test for the absence of endogenous interference, the internal standard was omitted for a blank plasma extract. No major endogenous peaks which would interfere with the resolution of the four compounds were encountered. In some samples, however, a slight interference corresponding to an adjacent endogenous component eluting close to albendazole was detected. This problem was readily eliminated by decreasing the ethanol concentration in the mobile phase to 450:50 (v/v). Furthermore, the peak mensuration technique (to the trailing edge extrapolated baseline) reduced any such interference.

Although the separation and subsequent estimation of benzimidazoles in plasma by reversed-phase high-performance liquid chromatography has been previously described [5, 6], the present method based on microparticulate silica permits the separation of albendazole and its metabolites in a one-step chromatographic run. Moreover, other benzimidazoles, such as oxfendazole and fendendazole, have been successfully separated from albendazole and its metabolites by means of this chromatographic system (Alvinerie and Galtier, unpublished results). A further advantage of the proposed method is in the use of an internal standard which has the requisite extraction, chromatographic and UV characteristics.

With regard to relative sensitivity, taking a signal-to-noise ratio of two as a criterion, the detection limits of the proposed method using the equipment described were found to be 20 ng/ml for albendazole, 40 ng/ml for albendazole sulphone and 50 ng/ml for albendazole sulphoxide based on a 100 μl plasma sample.

These data compare well with the detection limits previously described [6] (albendazole, 20 ng/ml; sulphone, 50 ng/ml; sulphoxide, 300 ng/ml). In particular, the higher sensitivity for albendazole sulphoxide, which represents the major therapeutically active metabolite [2], is a significant improvement. The sensitivity of the proposed method could be enhanced by increasing the amount of sample, thus permitting the accurate measurement of concentrations as low as 10 ng/ml.

Preliminary kinetic study

The plasma concentrations of albendazole and its two metabolites are illustrated in Fig. 3. These results are in good agreement with these previously obtained [6]. Albendazole levels decreased rapidly so that 1 h after administration the concentration of unchanged drug in plasma was below the limit of detection (0.02 μg/ml). Albendazole sulphoxide and sulphone peaked in plasma at respectively 1.5 and 8 h after administration. The parent drug is rapidly metabolized to the sulphoxide and sulphone, probably by a liver microsomal oxidation mechanism [6]. Preliminary *in vitro* studies carried out by the authors are in agreement with this suggestion. The later appearance of

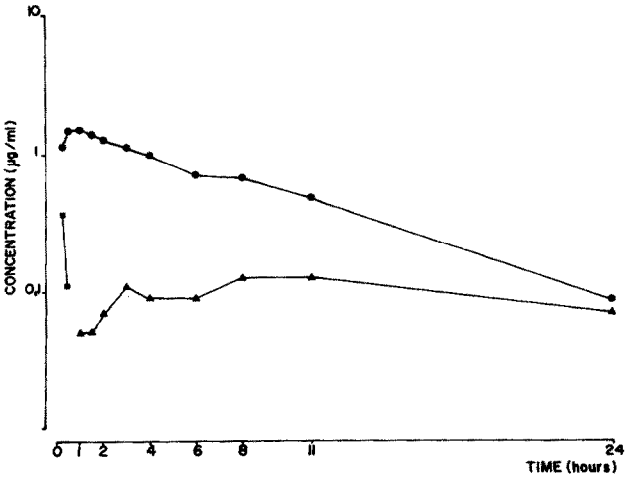

Figure 3
Plasma levels of albendazole and the sulphoxide and sulphone metabolites after intravenous administration of 1.9 mg/kg of albendazole in a sheep (albendazole ■, sulphoxide ●, sulphone ▲).

the sulphone metabolites compared with the sulphoxide would be related to the fact that this derivative is the terminal product of metabolism appearing in plasma [3].

Furthermore, it has been recently demonstrated [3] that albendazole is eventually metabolized to the amino-sulphone, which has been only recovered in the urine of dosed animals. Although this metabolite was not found in this experiment, it was confirmed that it did not interfere in the assay. The proposed method appears to be efficient for pharmacokinetic studies of albendazole and is currently being used for monitoring plasma levels during oral, intravenous and intra-ruminal experiments in sheep.

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